Wt1 is required for mesenchymal cardiovascular progenitor formation through direct transcriptional control of *Snail* and *E-cadherin*

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Supplementary Information

Supplementary Figure 1. Diagrammatic representation of ES cell targeting strategy. (a) Shows the endogenous wild type locus, with the position of Exon 1 marked within two PstI restriction sites. The position of the 3' probe used in Southern blotting to analyse clones and the genotyping PCR primers are also indicated. P= PstI restriction enzyme site (b) depicts the completed targeting vector. Exon 1 is flanked by loxP sites (black triangles) and a neomycin cassette surrounded by frt sites (open triangles) has been inserted. The lengths of the homology arms are shown. The NotI (N) sites used to isolate the insert are derived from the vector backbone. (c) Shows the locus after homologous recombination with the sites of the two probes used to confirm correct targeting marked. The lengths of the bands detected by the southern probes are also shown. (d), displays the 'conditional allele', the result recombination brought about by transient expression of FLPe recombinase where the neomycin selectable marker is deleted and only one Frt site remains. These are the cells which were injected into blastocysts to generate chimeras. The position of the genotyping PCR across the 5' loxP site is indicated. To verify that the targeted locus behaves as expected, a vector expressing the Cre recombinase was transfected. As shown in (e), this removes Exon 1 leaving behind only one loxP site. Southern blotting to confirm correct structure of the targeted locus. (f) shows the results of Southern blotting genomic DNA isolated

from wild type ES cells and one of the targeted cell lines after various steps (initial targeting, FLPe expression and FLPe followed by Cre expression). The DNA was digested with PstI and probed with the 3' probe. In all lanes the same wildtype 6.8kb band is detected while the band from the targeted allele alters reflecting the alteration in locus structure; from 2.9kb after initial targeting to 4.6kb after FLPe expression to 2.95kb after subsequent expression of Cre.

(g) shows the results of a genotyping PCR on a litter of progeny from an intercross of parents carrying the conditional allele. The primers cross the 5' loxP site and amplify a wildtype band of 198bp while the mutant band is 40bp bigger, reflecting the inclusion of a PstI restriction site and a loxP site. M= molecular weight marker; Wt= wildtype; Het= heterozygous for the conditional allele; Hom= homozygous for the conditional allele.

Supplementary Figure 2. Generation of epicardial-specific *Wt1* mutant mice. Immunofluorescence on sections of control (*Cre*$^{-}$) and mutant heart (*Cre*$^{+}$) with antibodies against Wt1. Wt1 is expressed in the epicardial cells of control embryos (*a, a'*), whereas its expression is deleted in epicardial cells from mutant hearts (b, b'). Scale bars represent 100µm. (c) RNA isolated from FACS-sorted GFP^{+} epicardial cells isolated from *Cre*$^{+}$ and *Cre*$^{-}$ mice was subjected to real-time PCR analysis. Wt1 expression was normalized to Gapdh expression. The graph represents the mean values ±s.e.m from three independent experiments.

Supplementary Figure 3. *Gata5-Cre* activity in E10.5 heart section visualized in Rosa26R Lacz reporter mice. We detected cells expressing *Gata5-Cre* in the epicardium. Scale bar represents 200µm.

Supplementary Figure 4. GFP expression on sections of E14.5 control and mutant hearts. The presence of GFP^+ epicardial cells covering the surface of the myocardium in Cre^+ mice demonstrate the integrity of the epicardium in mutant mice. Scale bars represent 100µm.

Supplementary Figure 5. Expression pattern of mesenchymal and epithelial proteins in Cre^- CoMEEC. (a) Western blot analysis for the indicated protein was conducted in Cre^- CoMEEC. (b) *In vitro* wound model in Cre^- CoMEEC in presence or absence of tamoxifen. We have not observed any difference in the markers that were analysed or in the migration properties of Cre^-CoMEEC after tamoxifen treatment.

Supplementary Figure 6. Snail is a repressor of the *Cdh1* gene in epicardial cells. *Cdh1* promoter construct was transfected in epicardial cells treated with *Snai1* shRNA and control shRNA lentiviral particles. *Snai1* knowdown in epicardial cells with a shRNA leads to an increase in *Cdh1* promoter activity. The graph represents the mean values ±s.e.m from three independent experiments.

Supplementary Figure 7. Model for the role of Wt1 in the generation of progenitor cells in the epicardium and ES cells differentiation. During heart development and ES cells differentiation, epicardial and ES cells undergo an EMT in order to produce mesenchymal progenitor cells that will generate mature differentiated cells. Wt1 is able to regulate this process thorough the direct activation of Snail and repression of E-cadherin.

Supplementary video. Movie showing a 3D OPT image of control (1) and mutant hearts (2). The coronary arteries failed to form in Wt1 mutant mice.

Table I

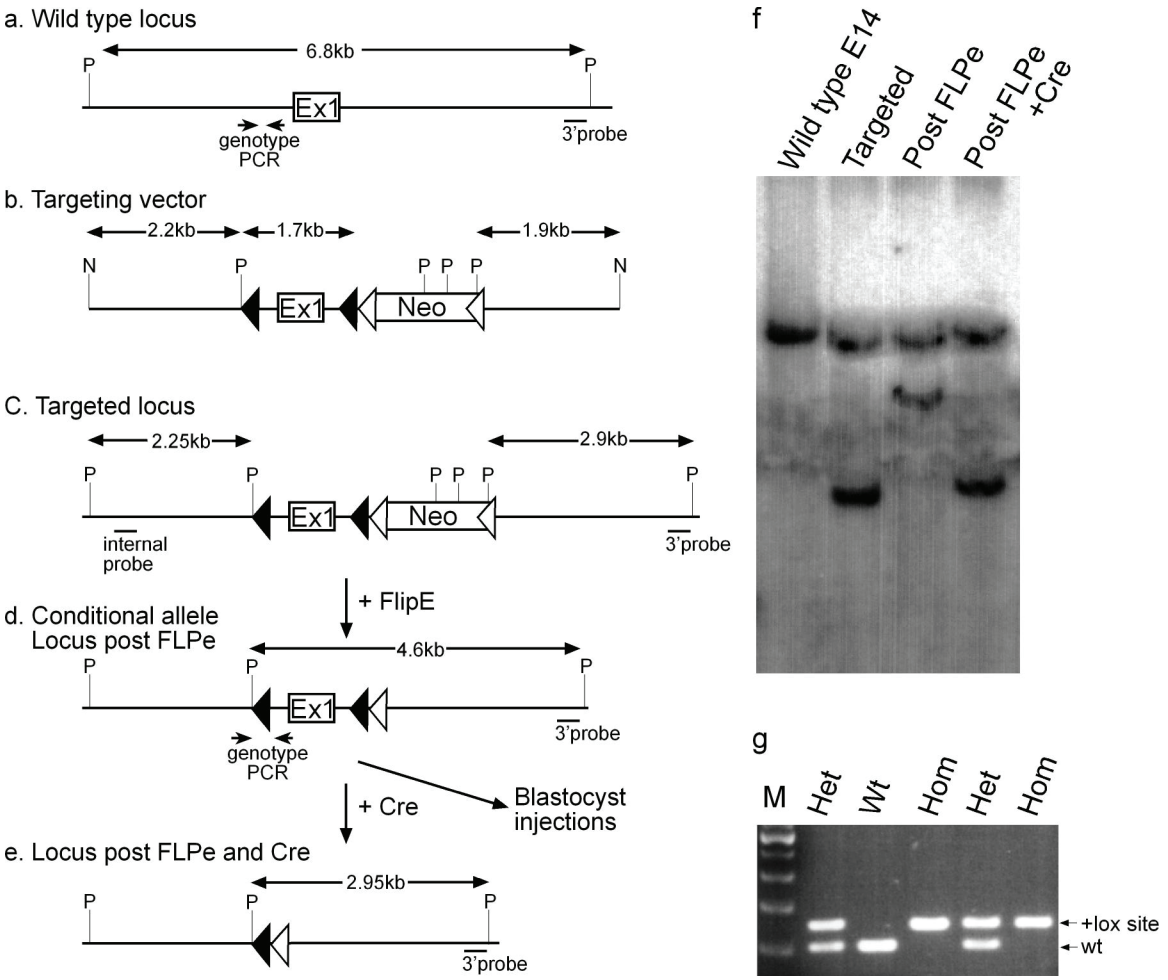
Primers:	**Genotyping**
Wt1 $^{loxP/loxP}$	TGGGTTCCAACCGTACCAAAG
	GGGCTTATCTCCTCCCATG
Wt1-GFP	GCCTGAAGAACGAGATCAGC
	GGCAGCTTGAATTCCTCTCA
	AGCCTGAAGCTGCTCACATC
Gata5cre	GCATTACCGGTCGATGCAACGAGTGATGAG
	GAGTGAACGAACCTGGTCGAAATCAGTGCG
Primers	**ChIP**
Mouse *Snai1* (promoter)	GCGCCCAAAGGTCAGCAGCTCG
	ACGCCCCCTTTGTCAAGGCTGAGC
Mouse *Snai1* (intronic)	TGTGTGTCTTAATGGTAGCCCAGG
	TCCTGGCAAGTGTGAAATCGGCA
Mouse *Snai1* (3'UTR)	ACACAGCTGCTTCGAGCCATAGAA
	ACATGTGTCCAGTAACCACCCT
Mouse Cdh1 (promoter)	TCAGAGCACAGCTAGGCTAGGATT
	CAGGAGTCTAGCAGAAGTTCTTGGGA
Mouse *Cdh1* (intronic)	ACAAGTGGGTGCTTGTCACAGAGT
	TTCTACAGAGGAAGAACACGAGGC
Mouse *Cdh1* (3'UTR)	TCATGTAATGTTGCTAGAGTGACCT
	TTGGTCTAGGAATCCTGGCAGAGT

Primers	**Mutagenesis**
Snai1 promoter	GGTCTCAGTCTCCGGCCACACCGCCGCC
Cdh1 promoter	CAGCGACACCGGGGGCGGTGCCTGCGGGCT
Cdh1 3'UTR	GTGTGTGAGTACTGATAATTTTGTATTTTGTGGGGGG
Primers	**RT-PCR**
AFP	CCTATGCCCCTCCCCCATTC
	CTCACACCAAAGCGTCAACACATT
T	ATGCTGCAGTCCCATGATAACTGG
	AACCAGAAGACGAGGACGTGGC
Cdh1	CGAGAGAGTTACCCTACATA
	GTGTTGGGGGCATCATCATC
Fgf5	TGTACTGCAGAGTGGGCATCGG
	ACTTCTGCGAGGCTGCGACAGG
Kdr	GCCCTGCTGTGGTCTCACTAC
	CAAAGCATTGCCCATTCGAT
Gsc	GAGCAGCTGGCCAGGAAGGTGCAC
	CAGCTAGCTCCTCGTTGCTTTCTC
Hand1	AAGACTCTGCGCCTGGCTACCA
	CGCCCTTTAATCCTCTTCTCGC
Hbb-b1	AAGGTGAACGCCGATGAAGTTGG
	TCATATTGCCCAGGAGCCTGAAG
Hnf4	TGCCCTCTCACCTCAGCAATG
	CCCCTCAGCACACGGTTTTG

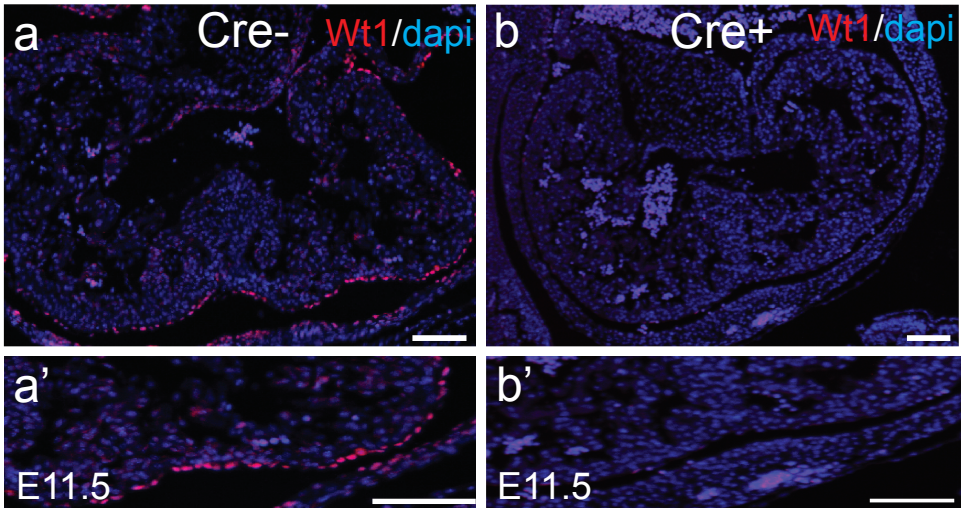
Isl1	AGCTGTACGTGCTTTGTTAGGGAT
	TCTTCTCGGGCTGTTTGTGGA
Nestin	CTCGAGCAGGAAGTGGTAGG
	TTGGGACCACCCACTGTTAG
Nkx2.5	GGCGTCGGGGACTTGAACACC
	CGCACTCACTTTAATGGGAAG
Snai1	CAGCTGGCCAGGCTCTCGGT
	GCGAGGGCCTCCGGAGCA
Snai2	GAATTCATGCCGCGCTCCTTCCTCGTCAAG
	GCGATCGCTCAGTGTGCCACACAGCAGCCA
Sox2	GAAGGGGAGAGATTTTCAAAGAGATACAAG
	CCAGATCTATACATGGTCCGATTCCC
Twist1	TGGACAGAGATTCCCAGAGG
	AAAATGGAGCCAGTCCACAG
Tek	AGTGTCTATACAACCAACAGTGATGTCTGG
	CACGTATGTCTTCCGTTCTTCCAG
Cdh5	CCTGACTGGAACCAGCACGCT
	GTGTGTCGTATGGGGGGCCAC
Wt1	ATCCTCTGTGGTGCCCAGTA
	CGACAGCTGAAGGGCTTTTC
Gapdh	GCCAAAAGGGTCATCATCTCTG
	CATGCCAGTGAGCTTCCCGT
Primers	**Real time-PCR**
Snai2	CATTGCCTTGTGTCTGCAAG

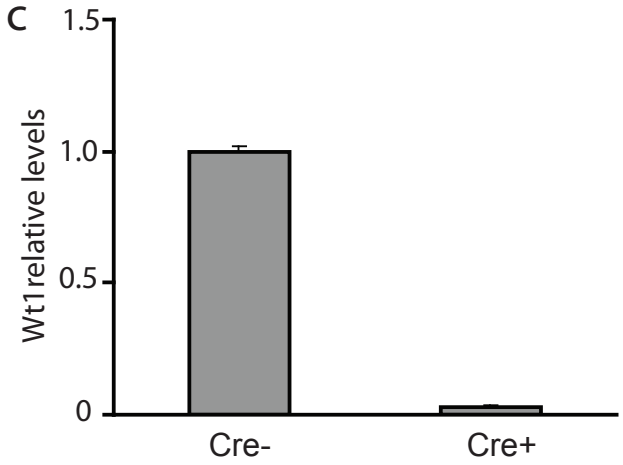
	CAGTGAGGGCAAGAGAAAGG
Snai1	CTTGTGTCTGCACGACCTGT
	CAGGAGAATGGCTTCTCACC
Wt1	CAGGATGTTCCCAATGC
	GAAAGTGACCGTGCTGTATCC
Gapdh	Gapdh gene assay, Roche cat no. 05046211001

Supplementary Fig. 1



Supplementary Fig. 2





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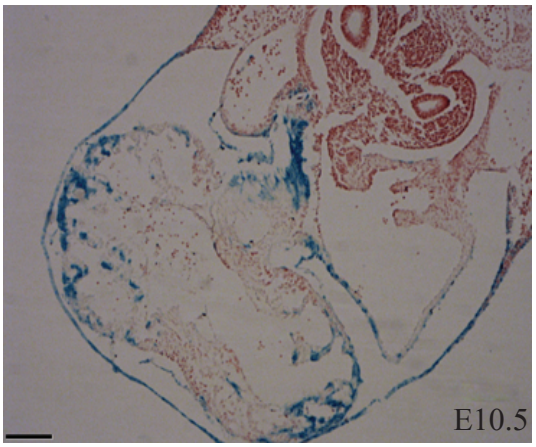

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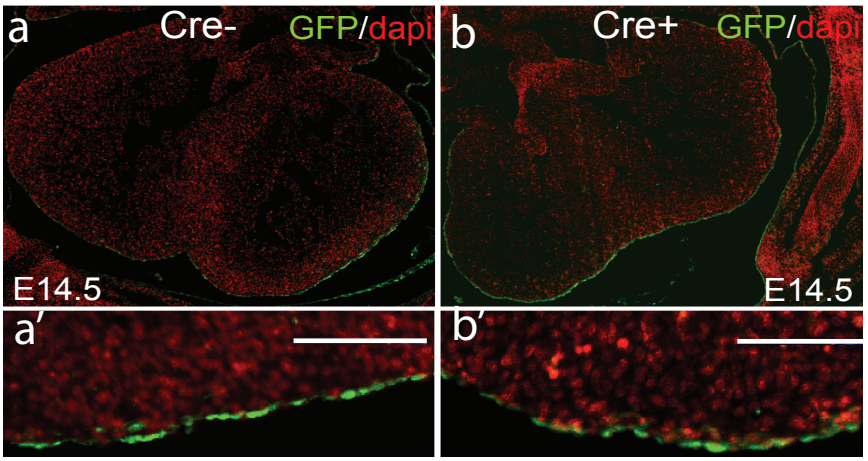

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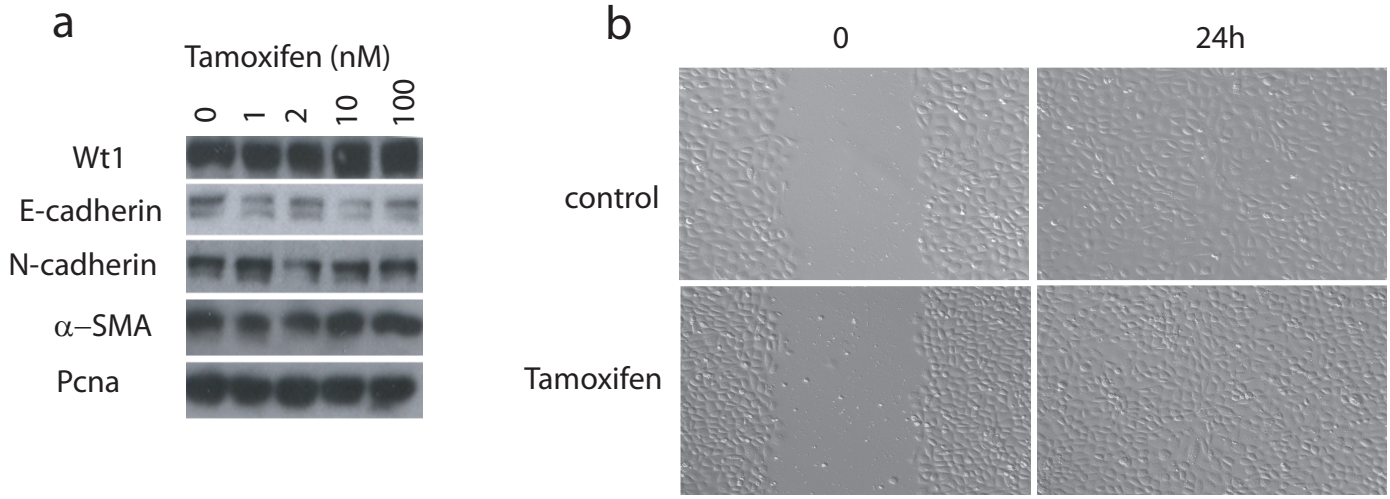

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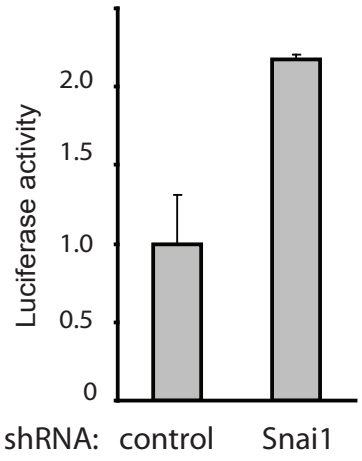

Supplementary Fig. 7